AN AUTOMATED METHOD FOR SEGMENTING HIGHLY CONVOLUTED MITOCHONDRIAL INNER MEMBRANES FROM ELECTRON MICROSCOPIC TOMOGRAMS

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ABSTRACT

The functional importance of the morphology of the mitochondrial inner membrane (IM) is demonstrated both by the complex machinery required to maintain normal morphology and by specific changes in shape associated with physiological and pathological states. Accurate computer simulations of activities such as ATP production and calcium uptake are best performed in 3-dimensional space using realistic membrane surfaces, such as those provided by transmission electron microscopic (TEM) tomography. However, the observed variability in IM shape requires sampling a wide range of crista morphologies (from tubular to lamellar in the case of cardiac mitochondria), and the complexity and compaction of cristae make segmentation a challenge. The traditional method of segmenting the TEM tomographic images involves manually tracing membrane contours in 2D slices from tomograms, a laborious technique that is generally more effective for less intricate membrane shapes. Thus, we are developing an automated methodology of segmentation that employs a novel reproducible sequence of procedures for membrane segmentation from TEM tomograms of epoxy-embedded and stained mouse cardiac muscle by Frangi enhancement filtering, a Hessian-based algorithm that has been used in medical imaging (ocular vasculature enhancement and mapping). We have found the new automated procedure to work well for segmenting intricate membranes against a noisy background. The next step is constructing an accurate mesh model of a membrane of interest using the MATLAB isosurface function. In the current implementation, the procedure does an excellent job of isolating individual membranes inside crowded mitochondria. However, there is still a need to manually correct spurious features, such as random breaks in membrane surfaces due to stain noise. Conditions are being explored to minimize or eliminate these, as are automated methods to correct discontinuities.

INTRODUCTION

The mitochondrial cristae are folds of the mitochondrial inner membrane containing protein complexes involved in energy metabolism. The adjacent diagram shows the different parts of a mitochondrion. In Figure 1A, the inner membrane (in oval) is shaped to maximize its surface area and the mitochondrial matrix is a viscous space within the inner membrane.

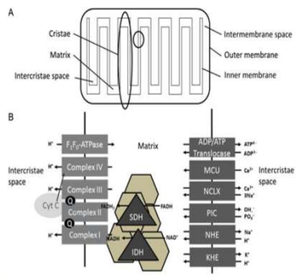


Figure 1. Mitochondrial schematic from Jafri and Kumar 2014

The circled region is enlarged in Figure 1B. This is the site for energy metabolism containing proteins for the respiratory chain and oxidative phosphorylation located in the membrane and the tricarboxylic acid (TCA) cycle. The rest are proteins involved in the transport of substances in and out of the mitochondria. (MCU, mitochondrial calcium uniporter; NCLX, mitochondrial sodium–calcium–lithium exchanger; PIC, phosphate carrier; NHE, sodium hydrogen exchanger; KHE, potassium hydrogen exchanger).

Inner membrane structure varies with cell type and functional state (Mannella, Lederer, and Jafri 2013). Since diseases are often associated with specific ultrastructural changes, understanding the structure of this organelle via 3D electron microscopy is crucial. The noisy nature of the data and disparities in membrane shapes has made it difficult to develop a general segmentation process applicable to all mitochondrial structures, but we have found a combination of methods that is promising.

Flowchart for Image Data Segmentation and Structure Rendering

Image Processing Algorithms

Image data stack from electron microscopy (EM) or computed tomography (CT) loaded into Microscopy Image Browser (MIB)

↓

Anisotropic- Denoise and smooths

↓

Frangi- Structural enhancement

↓

Median- Smooths and preserves edges

Segmentation and Structure Rendering (can use ML or AI)

Select a feature or object from image data using MIB

↓

Region of Interest (ROI)- Selection and interpolation

↓

Thresholding- Automated extraction of features

↓

Smooth the object and build the model

↓

Save the structure or object

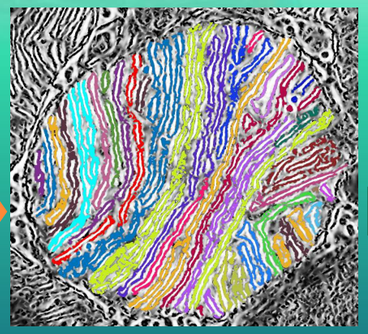


Figure 3. Segmented cristae.

Comparison of Image Enhancement

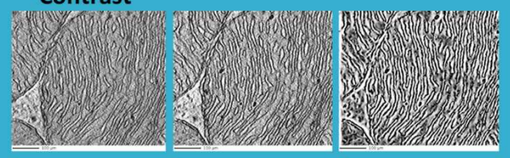


Figure 2. Comparison of enhancement methods. The first column utilizes auto contrast; the second column underwent anisotropic filtering; and the third column displays Frangi enhancement.

The initial step is capturing the mitochondria in high resolution. This is done by transmission electron microscopy (TEM). As of now, human judgment is still necessary to analyze and select the means of method for selecting the mitochondria being segmented. This study used Matlab and Microscopy Image Browser (MIB) as a segmentation platform. MIB is a software suite for high-performance segmentation and image processing of multidimensional datasets. Figure 2 Illustrates the preprocessing of the image volume. Figure 2A is the original image with an adjusted contrast. Figure 2B is the same image slice with anisotropic diffusion applied. The anisotropic diffusion is also called Perona-Malik diffusion. It eliminates noise by using an impulse function while retaining significant features of the image. Frangi filter is then used in Figure 2C. This is a Hessian-based method developed by Frangi and his group in 1999. To further eliminate noise, a median filter was used.

Region of Interest (ROI)

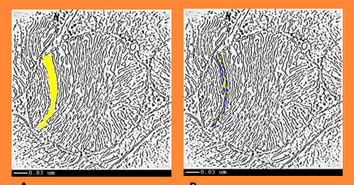
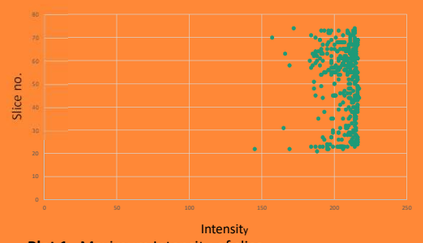


Figure 4. Selection and thresholding of images.

Maximum Intensity



Plot 1. Maximum Intensity of slice.

Figure 4 demonstrates the highlighting and thresholding of different regions. Figure 4A and 4B are region 1 of this mitochondrion. This region has cristae that are very close together. The ROI was selected before thresholding. The thresholding method automatically traces each feature of this region (Figure 4A). Figure 3 is the completed segmentation of the cardiac mitochondria. Plot 1 shows the maximum intensity of the segmented areas of each slice. MATLAB isosurface is then used to build the mesh.

RESULTS

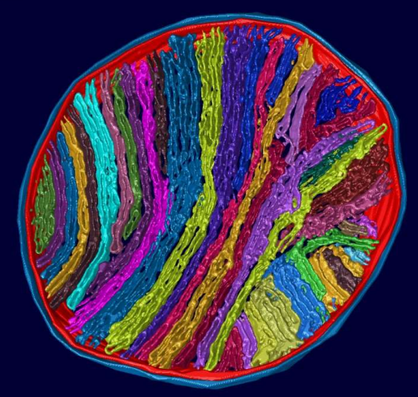


Figure 5. 3D isosurface of mitochondrial inner membranes

Generated Mesh of 4 Cristae

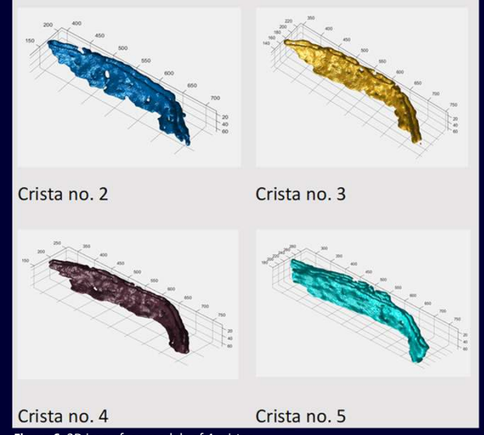


Figure 6. 3D isosurface models of 4 cristae.

CONCLUSION

Whole mitochondrion can be done all at once using thresholding, but it does not always provide the necessary features and accuracy though it may be useful for studying measurements, basic shapes and structures, protein localization, and compartmentalization. As shown on Figure 6, rendering the cristae by regions provides a better understanding of how one crista connect to the next. It also shows a more refined rendition of the features, such as curvature and fenestrations within the sides of the crista giving a better understanding of how the matrix connect from one region to another. In addition, taking 3D parts of the cristae will be more suitable for simulations and other computational experiments. Simplification can be done by simulations using individual crista or simplifying the number of polygons to simplify the shape.

Due to the size and complexity of cardiac mitochondria and tediousness of manual tracing, **traditional methods to segment all the membranes could take over a month**. It is likely for this reason that no segmented 3D model has been produced of a complete cardiac mitochondrion to date. **Our method takes days** (at the current stage) and has produced the **first completed segmentation of cardiac mitochondrial cristae.**

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Acknowledgments

This project is funded by the NIH - NHLBI grant U01HL116321